ORIGINAL ARTICLE

Safety and Immunogenicity of Adenovirus 35 Tuberculosis Vaccine Candidate in Adults with Active or Previous Tuberculosis

A Randomized Trial

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Abstract

Rationale: Administration of tuberculosis (TB) vaccines in participants with previous or current pulmonary TB may have the potential for causing harmful postvaccination immunologic (Koch-type) reactions.

Objectives: To assess the safety and immunogenicity of three dose levels of the AERAS-402 live, replication-deficient adenovirus 35–vectored TB candidate vaccine, containing three mycobacterial antigens, in individuals with current or previous pulmonary TB.

Methods: We performed a phase II randomized, placebo-controlled, double-blinded dose-escalation study in an HIV-negative adult South African cohort (n = 72) with active pulmonary TB (on treatment for 1–4 mo) or pulmonary TB treated at least 12 months before study entry and considered cured. Safety endpoints included clinical assessment, flow volume curves, diffusing capacity of the lung for carbon monoxide, pulse oximetry, chest radiograph, and high-resolution thoracic computerized tomography scans. Cytokine expression by CD4 and CD8 T cells, after stimulation with Ag85A, Ag85B, and TB10.4 peptide pools, was examined by intracellular cytokine staining.

Measurements and Main Results: No apparent temporal or dose-related changes in clinical status (specifically acute, Koch phenomenon–like reactions), lung function, or radiology attributable to vaccine were observed. Injection site reactions were mild or moderate. Hematuria (by dipstick only) occurred in 25 (41%) of 61 AERAS-402 recipients and 3 (27%) of 11 placebo recipients, although no gross hematuria was reported. AERAS-402 induced robust $CD8^+$ and moderate $CD4^+$ T-cell responses, mainly to Ag85B in both vaccine groups.

Conclusions: Administration of the AERAS-402 candidate TB vaccine to participants with current or previous pulmonary TB induced a robust immune response and is not associated with clinically significant pulmonary complications.

Clinical trial registered with www.clinicaltrials.gov (NCT 02414828) and in the South African National Clinical Trials Register (www.sanctr.gov.za DOH 27-0808-2060).

Keywords: tuberculosis; TB vaccine; vaccine safety; pulmonary complications; vaccine immunogenicity

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At a Glance Summary

Scientific Knowledge on the Subject: Tuberculosis (TB) vaccines are being developed to assist the control of TB worldwide. Vaccines aim to enhance immunologic defenses but if inadvertently administered to persons with currently or previously treated pulmonary TB, might potentially result in the Koch phenomenon and further damage to the lung. In this study, the AERAS-402 candidate vaccine administered in increasing doses to two groups of participants (one previously treated, and the other currently receiving treatment, for pulmonary TB) resulted in robust mycobacterial antigen–specific immune responses, and was not associated with any clinically significant adverse effects on clinical or radiologic status or lung function deterioration.

What This Study Adds to the Field: The AERAS-402 adenovirus serotype 35 replication-deficient vaccine containing DNA encoding for mycobacterial antigens 85A, 85B, and TB10.4 when administered to participants currently or previously treated for pulmonary TB is not associated with clinically significant adverse systemic or pulmonary effects, suggesting that TB vaccination with this vector may be safe in populations in which TB vaccines may be inadvertently administered to persons with active or previous pulmonary TB.

Tuberculosis (TB) control remains a major global challenge despite the availability of effective chemotherapy. The World Health Organization End TB strategy has set targets of 90% reduction in incidence by 2035 and TB vaccination is an integral part of this strategy in addition to early detection and treatment of *Mycobacterium tuberculosis* (*M.tb*) (1, 2). To date, the search for an effective vaccine has had limited success.

Current efforts by Aeras to develop a TB vaccine focus on products that prevent active disease among older children and adults latently infected with *M.tb* (3). In high-prevalence TB countries, TB vaccines may inadvertently be administered to persons with active or recently treated TB, with the potential for an exaggerated immunologic response: inflammation and necrosis at sites of antigen exposure (the Koch phenomenon, observed in whole-cell mycobacterial vaccines) (4); systemic manifestations (chills, fever, and malaise); and, in extreme cases, even shock and death from a "cytokine storm" (5, 6).

AERAS-402 is a replication-deficient, adenovirus serotype 35 (Ad35) containing DNA encoding a fusion protein created from the sequences of three *M.tb* antigens: Ag85A, Ag85B, and TB10.4 (7). In animal models these antigens have been shown to induce protective immunity when presented in recombinant viral vectors (8). Specifically, intranasal and intramuscular administration of AERAS-402 in mice conferred protective immunity against *M.tb* (9). However, it failed to protect, despite robust cellular immune responses, against high-dose *M.tb* challenge in macaques (10). In an earlier, uncontrolled clinical study, AERAS-402 was well tolerated at single doses of 3×10^8 viral particles (vp) and 3×10^9 vp, and two doses of 3×10^{10} vp, in Bacillus Calmette-Guerin–unvaccinated healthy adults. No safety concerns were identified but all participants enrolled were free from latent or active TB infection. Safety in those with current TB infection had not been tested. Measurable $CD8^+$ and $CD4^+$ T-cell responses to the candidate transgene antigens have been demonstrated (11, 12).

We report here the results of Study C-010-402, examining the safety and immunogenicity of three dose levels of AERAS-402 in participants with active TB on treatment or previously treated TB. Some of the results have been presented in abstract form (13–15).

Methods

Study Design

C-010-402 was a double-blind, randomized, placebo-controlled dose-escalation study of AERAS-402 in 72 adults negative for HIV stratified into two groups: participants currently being treated (with signs of clinical improvement) for active pulmonary TB (APTB) between 1 and 4 months before Day 0; and participants considered cured post-treatment for pulmonary TB (PPTB), who started TB treatment at least 12 months before Day 0. Participants were randomized between October 29, 2008, and December 9, 2009, to receive placebo or candidate vaccine in one of three dosage regimens: a single dose of 3×10^8 vp (group 1) or 3×10^9 vp (group 2), or two doses of 3×10^{10} vp (group 3) administered intramuscularly on Days 0 and 42 (Figure 1). Dose escalation within a treatment stratum occurred only after safety data for the previous dose through Day 28 had been reviewed and no serious adverse events (AEs) or other major safety concerns were identified. No participants were enrolled in the planned group 4 (1×10^{11} vp). No safety concerns had been identified, but, after a review of available resources and priorities, the sponsor and their partner (Crucell) decided not to enroll group 4. The final follow-up visit was on July 5, 2010. The full protocol is available in the online supplement.

Safety Evaluation

Safety evaluations were performed on Days 0, 1, 2, 7, 14, 28, 42, 84, and 182 in all participants, and Days 43, 44, 49, 56, and 70 in participants receiving two doses (Figures 2A and 2B). Unsolicited and solicited AEs (response to specific questions on symptoms potentially related to vaccination, such as injection site reactions, and to its potential effect on pulmonary TB [*see* online supplement]) were collected from vaccination through 28 days after each vaccination, and serious AEs to Day 182.

Chest radiographs (CXRs) were performed on Days 7, 14, 42, 84, and 182 in all groups and additionally on Days 49 and 56 in group 3. Low-dose high-resolution chest computed tomography (CT) scans were performed on Days 0 and 28 in all groups, and also on Day 70 in group 3. Lung function tests, including FEV_1, FVC (16, 17), and diffusing capacity of the lung for carbon monoxide (D_{LCO}) were performed at each study visit (except at 1 and 2 days after vaccination), according to American Thoracic Society standards (16, 18). Detailed methods for lung function and imaging are in the online supplement.

Immunogenicity and Related Assessments

Immune responses were measured by flow cytometry in a seven-color intracellular cytokine staining (ICS) on peripheral blood

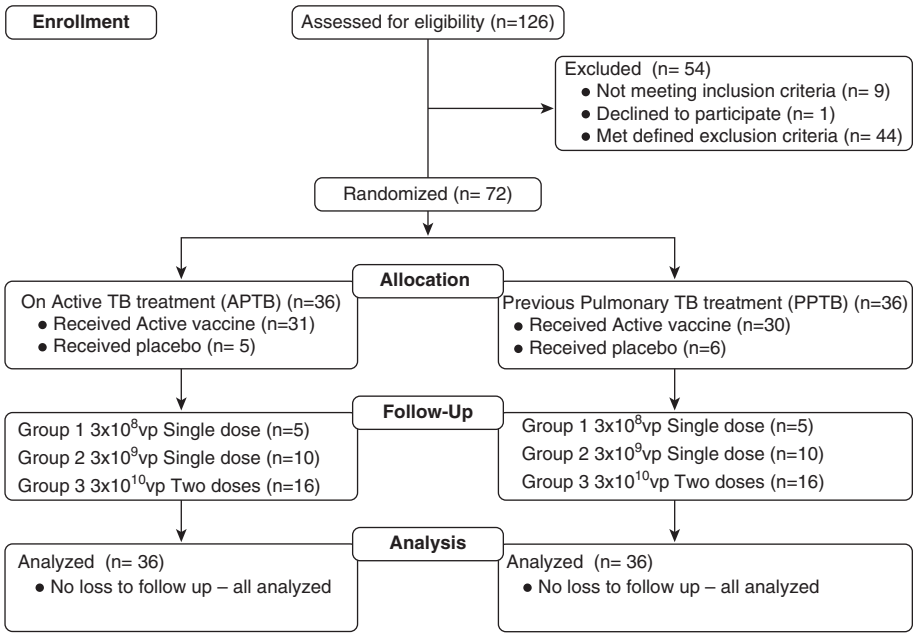

Figure 1. Study flow (Consolidated Standards of Reporting Trials diagram) of patient allocation and randomization to vaccine and placebo. All patients were evaluated in the acute safety follow-up with no loss to follow-up. TB = tuberculosis; vp = viral particles.

mononuclear cells (PBMCs) or an eight-color whole-blood ICS assay. Immune responses were measured as the percentage of $CD4^+$ and $CD8^+$ T cells that produced any of IFN-γ, tumor necrosis factor (TNF)-α, and/or IL-2, alone or in combination after stimulation with peptide pools spanning the entire amino acid sequences of Ag85A, Ag85B, and TB10.4. Detailed methods are presented in the online supplement.

Statistics

Basic descriptive statistics were performed to analyze AEs, lung parameters, and ICS assay results. All participants who received at least one dose of the candidate vaccine or placebo were included in AE analyses, tabulated by MedDRA version 11.1 (McLean, VA) preferred term. In addition, *post hoc* analyses were performed on immunogenicity data. Full details are contained in the online supplement.

Ethics

The University of Cape Town Human Research Ethics Committee and the Chesapeake Institutional Review Board (for Aeras) approved the study. Participants provided informed consent before enrollment.

Results

Participants

Seventy-two participants were enrolled, 36 in each of the APTB and PPTB strata. Five participants in the ATPB stratum and six in the PPTB stratum were randomized to placebo. The patient characteristics are presented in Table 1.

A larger proportion of PPTB participants than APTB reported current or previous smoking (Table 1) (78% vs. 64%). Self-reported ethnicity of all but two participants was either colored (mixed race) or African black (data not shown). No differences in demographic characteristics among the three candidate vaccine dose groups, or between recipients of placebo and the vaccine groups, in either the APTB or PPTB strata were apparent (data not shown).

Adverse Events

Thirty-six (100%) participants in the APTB stratum, and 35 (97%) participants in PPTB stratum reported at least one AE during the study. No deaths occurred during the study period. A record of all AEs, solicited, unsolicited, and those considered to be TB-associated, categorized by candidate vaccine dose and System Organ Class in the APTB stratum and PPTB stratum, respectively, are presented in Tables E1 and E2 in the online supplement. Only those for which there was a greater than 20 percentage point difference between vaccine and placebo recipients for the APTB and PPTB strata (generally representing a difference between groups of one or two subjects) are presented in Table 2. The injection site reactions, all mild or moderate, increased with increasing AERAS-402 dose level. Disparity between AERAS-402 and placebo for D_{LCO} occurred only at the highest dose level in the APTB stratum; however, all events were mild and considered unlikely to be vaccine-related. In addition, trends in mean D_{LCO} over time did not show any effect of AERAS-402 at the two higher dose levels (*see* Lung Function section).

Hematuria detected by urine dipstick was present in 25 (41%) of 61 vaccine recipients (all at the highest two dose levels) and 3 (27%) of 11 placebo recipients (data not shown). There was disparity between dipstick and microscopic examination; dipstick positivity was confirmed by microscopy in 19 of 25 cases in vaccine recipients and two of three cases in placebo recipients. There were no reports of gross hematuria, or correlation with development of Ad35 neutralizing activity.

Fever was reported in 5 (8%) of 61 vaccine recipients (four in the high-dose group and one receiving the middle dose), one of which was severe (*see* later); the remaining four were mild. No fever was reported among placebo recipients.

Severe AEs were more common in the APTB stratum; 7 (23%) of 31 vaccine recipients had eight severe AEs (there were no severe AEs in placebo recipients) (*see* Table E3). These included one solicited AE of pyrexia (39.0°C) 2 days after the first vaccination with 3×10^{10} vp, considered possibly vaccine-related; it resolved 2 days later and did not recur after the second dose of vaccine. One participant who received 3×10^8 vp had bronchioalveolar carcinoma and died 10 months later, after study end. A causal relationship to vaccine is not suspected; review of radiographs suggested its presence at the time of enrollment. The remaining severe AEs, all considered unlikely to be vaccine-related, were urine abnormalities in three participants who received 3×10^9 vp and one participant who received 3×10^{10} vp (glycosuria, red blood cells, proteinuria, and both red blood cells and proteinuria in one participant), and decreased neutrophil

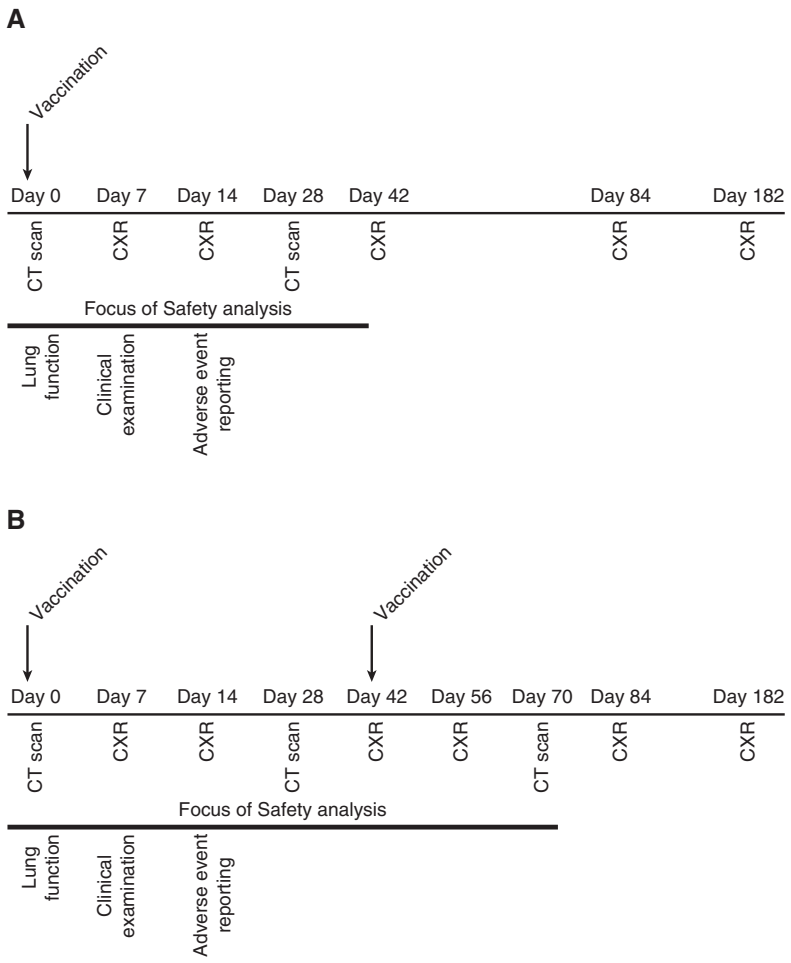

Figure 2. Study plan over 6-month period. (*A*) Groups 1 and 2 (one vaccination with 3×10^8 vp or with 3×10^9 vp, respectively). (*B*) Group 3 (two vaccinations with 3×10^{10} vp). Safety evaluations were performed on Days 0, 1, 2, 7, 14, 28, 42, 84, and 182 in all participants, and Days 43, 44, 49, 56, and 70 in participants receiving two doses. The *bold line* indicates the intensive safety follow-up period (clinical, lung function, and immunology) of this study. Time points for radiology are depicted for each stratum. CT = computed tomography; CXR = chest radiograph; vp = viral particles.

count in one participant who received 3×10^9 vp. The hematuria was microscopic (positive dipstick and microscopy) and none of the participants reported urinary symptoms or had historical or clinical evidence of renal or urinary tract disease.

In the PPTB stratum, no solicited or urine-related severe AEs were recorded. Three (10%) of 30 vaccine recipients reported four severe AEs (gamma-glutamyl transferase increased and labile blood pressure [3×10^9 vp], and gastric ulcer and decreased hemoglobin in one participant [3×10^{10} vp]), and one (20%) of five placebo recipients reported one severe AE (increased systolic blood pressure). All of these severe AEs were considered unlikely to be vaccine-related. The participant with gastric ulcer died 26 months after receiving AERAS-402, after the end of the study. None of the severe AEs in either the APTB or PPTB stratum resembled a Koch phenomenon reaction.

Lung Function

The proportions of subjects that showed deterioration in PFT parameters (>10% decrease from baseline for FEV_1 or FVC, or >15% decrease from baseline for D_{LCO}) are presented in Table E4 (for the APTB stratum) and Table E5 (for the PPTB stratum). Because of the small size of the groups, low frequency of changes, and lack of discernible trends, only time points showing the highest proportion of subjects with deterioration in each treatment group are presented in these tables. There was no apparent effect of AERAS-402 on the proportion of subjects with decreases from baseline in FEV_1, FVC, or D_{LCO}.

Trends for values of FVC and D_{LCO} by treatment group over time (Days 0–42) are presented for the APTB stratum in Figure 3A and for the PPTB stratum in Figure 3B. Data from participants receiving the middle- and high-dose levels of AERAS-402 (3×10^9 and 3×10^{10} vp) were pooled to increase the robustness of the analysis. In the APTB stratum, no clinically significant worsening in FVC was observed at any time during the postvaccination period. The apparent downward trend in D_{LCO} observed in the group that received the lowest dose of vaccine (n = 5) was not evident in the higher dose groups (n = 26), suggesting random variability in a small data set. In the PPTB stratum, there was no evidence of worsening of FVC and D_{LCO} during the study.

Radiography

CXRs at each time point were compared with the baseline CXR taken at Day 7. In the APTB stratum, 29 (94%) vaccine recipients and all five (100%) placebo recipients showed either no change or an improvement from Day 7 to Day 14 (Table 3). In the PPTB stratum, 26 (96%) vaccine recipients and all six (100%) placebo recipients showed no change in their CXRs from Day 7 to Day 14. Three chest radiographs showed worsening at Day 14, all in vaccine recipients. One participant in the high-dose group (APTB stratum) had worsening on CXR at 14 days after the second dose, but none deteriorated between days 84 and 182. In the APTB stratum, either no change or improvement on chest CTs from Day 0 to Day 28 was observed in 26 (93%) of 28 vaccinated and four (80%) of five placebo recipients (Table 4). In the PPTB stratum, 29 (97%) of 30 vaccinated and all six (100%) placebo recipients were unchanged or improved over this period. Worsening on chest CT was reported in three vaccine recipients (two in the APTB stratum and one in the PPTB stratum) at Day 28. Three participants in the high-dose group (two in the APTB stratum, one in the

Table 1. Baseline Characteristics

	APTB Stratum (*n* = 36)	PPTB Stratum (*n* = 36)
Age, yr, median (range)	28.0 (19–45)	28.5 (19–45)
Participants receiving vaccine	31	30
Participants receiving placebo	5	6
Sex (males), n (%)	19 (53)	22 (61)
FVC % predicted, mean (SD)	74.1 (16.6)	75.0 (16.7)
FEV_1/FVC %, mean (SD)	82.4 (9.0)	80.6 (10.3)
D_{LCO} % predicted, mean (SD)	66.6 (12.6)	72.5 (13.8)
History of smoking, no. (%) of patients	23 (64)	28 (78)

Definition of abbreviations: APTB = active pulmonary tuberculosis; D_{LCO} = diffusing capacity of the lung for carbon monoxide; PPTB = post-treatment for pulmonary tuberculosis.

PPTB stratum) had worsening chest CTs at 28 days after the second dose of vaccine. The CXR and chest CT worsening included enlarging or new cavities and/or increasing nodularity. In one participant (PPTB stratum), a recurrence of bacteriologically confirmed pulmonary TB was diagnosed. An additional patient (APTB stratum) was confirmed to have extensive bronchioalveolar carcinoma, accounting for the worsening. No lymphadenopathy was detected.

Immune Response Assays

Immunogenicity of AERAS-402 was measured by seven-color PBMC ICS assay on Days 0, 28, and 42 for groups 1 and 2 and Days 0, 28, 42, 70, and 84 for group 3. The results show a dominant $CD8^+$ T-cell response to Ag85B and to a lesser extent Ag85A, with a very weak response to TB10.4 after a single dose of AERAS-402 (Figure 4). Interestingly, a single dose with AERAS-402 at 3×10^9 vp gave higher, although only marginally statistically significant, total median $CD8^+$ responses to Ag85B on Day 28 (0.397%) compared with a single dose of AERAS-402 at 3×10^8 and 3×10^{10} vp (0.055% and 0.148%, respectively; $P = 0.109$ and $P = 0.068$) (Figure 4E). Similar trends were observed for $CD8^+$ responses against Ag85A; however, they were not statistically significant (group 1 vs. group 2, $P = 0.50$; group 2 vs. group 3, $P = 0.09$) (Figure 4D). Group 2 and group 3 each exhibited increased median $CD4^+$ immune responses after baseline (Ag85A, $P = 0.0002$ and $P < 0.0001$, respectively; Ag85B, $P = 0.0009$ and $P < 0.0001$, respectively). All three groups demonstrated increased median $CD8^+$ immune responses (Ag85A, $P = 0.002$, $P < 0.0001$, and $P < 0.001$, respectively; Ag85B, $P = 0.0014$, $P < 0.0001$, and $P < 0.0001$, respectively), with the increases most notable among recipients of a single dose of AERAS-402 at 3×10^9 vp (group 2) and two doses of AERAS-402 at 3×10^{10} vp (group 3). A second dose of AERAS-402 at 3×10^{10} vp significantly boosted total median $CD8^+$ responses against Ag85B from 0.143% on Day 42 to 0.670% on Day 84 ($P = 0.0034$) (Figure 4E). Polyfunctional analysis of the $CD8^+$ Ag85B response at Day 42 revealed similar functional profiles regardless of dosage, with primarily polyfunctional (IFN-γ, IL-2, and TNF), bifunctional (IFN-γ and TNF), and monofunctional (IFN-γ) responses to AERAS-402 (Figure 5A). A second dose of AERAS-402 at the highest dose seemed to bias the response more toward terminal effector cells, with a strong preference for bifunctional (IFN-γ and TNF) and monofunctional (IFN-γ or TNF) T cells with a weaker polyfunctional response (Figure 5B).

The whole-blood ICS assay confirmed robust antigen-specific $CD8^+$ T-cell responses to 3×10^9 and 3×10^{10} vp, but not the 3×10^8 dose. Responses to Ag85A and B and TB10.4-specific $CD4^+$ T cells were similar (*see* Figures E1 and E2). Notably, these T-cell responses were sustained, remaining above prevaccination levels up to Day 182 (*see* Figures E1 and E2). There was no discernible difference in the magnitude or quality of the immune responses in the APTB versus the PPTB stratum (data not shown).

Table 2. AEs with >20 Percentage Point Difference between Placebo and AERAS-402

	Placebo	Group 1	Group 2	Group 3
APTB stratum	n = 5	n = 5	n = 10	n = 16
Participants with at least one AE	5 (100.0)	5 (100.0)	10 (100.0)	16 (100.0)
Solicited AEs				
Injection site pain	1 (20.0)	1 (20.0)	5 (50.0)	11 (68.8)
Injection site erythema	1 (20.0)	1 (20.0)	3 (30.0)	7 (43.8)
Unsolicited AEs				
D_{LCO} decreased	0 (0.0)	0 (0.0)	0 (0.0)	6 (37.5)
PPTB stratum	n = 6	n = 5	n = 10	n = 15
Participants with at least one AE	6 (100.0)	5 (100.0)	10 (100.0)	14 (93.3)
Solicited AEs				
Injection site pain	0 (0.0)	0 (0.0)	2 (20.0)	9 (60.0)
Injection site swelling	0 (0.0)	0 (0.0)	2 (20.0)	4 (26.7)
Unsolicited AEs				
Protein urine	2 (33.3)	3 (60.0)	1 (10.0)	7 (46.7)
Hemoglobin decreased	1 (16.7)	2 (40.0)	2 (20.0)	6 (40.0)
Neutrophil count increased	0 (0.0)	0 (0.0)	0 (0.0)	4 (26.7)
White blood cell count increased	0 (0.0)	2 (40.0)	0 (0.0)	3 (20.0)

Definition of abbreviations: AE = adverse event; APTB = active pulmonary tuberculosis; D_{LCO} = diffusing capacity of the lung for carbon monoxide; PPTB = post-treatment for pulmonary tuberculosis.
Data are shown as n (%).

Ad35 Serum Neutralizing Activity

Eighteen (25%) of all participants had Ad35 serum neutralizing activity greater than or equal to the lower limit of quantitation at Day 0. Of the 46 vaccine participants who had activity less than the lower limit of quantitation at Day 0 (and data at Day 84), 19 participants (41.3%), 16 in the highest-dose group and three in the middle-dose group, developed Ad35 serum neutralizing activity by Day 84.

Discussion

This study is the first to examine the safety, tolerability, and induced immune responses of the AERAS-402 TB candidate vaccine

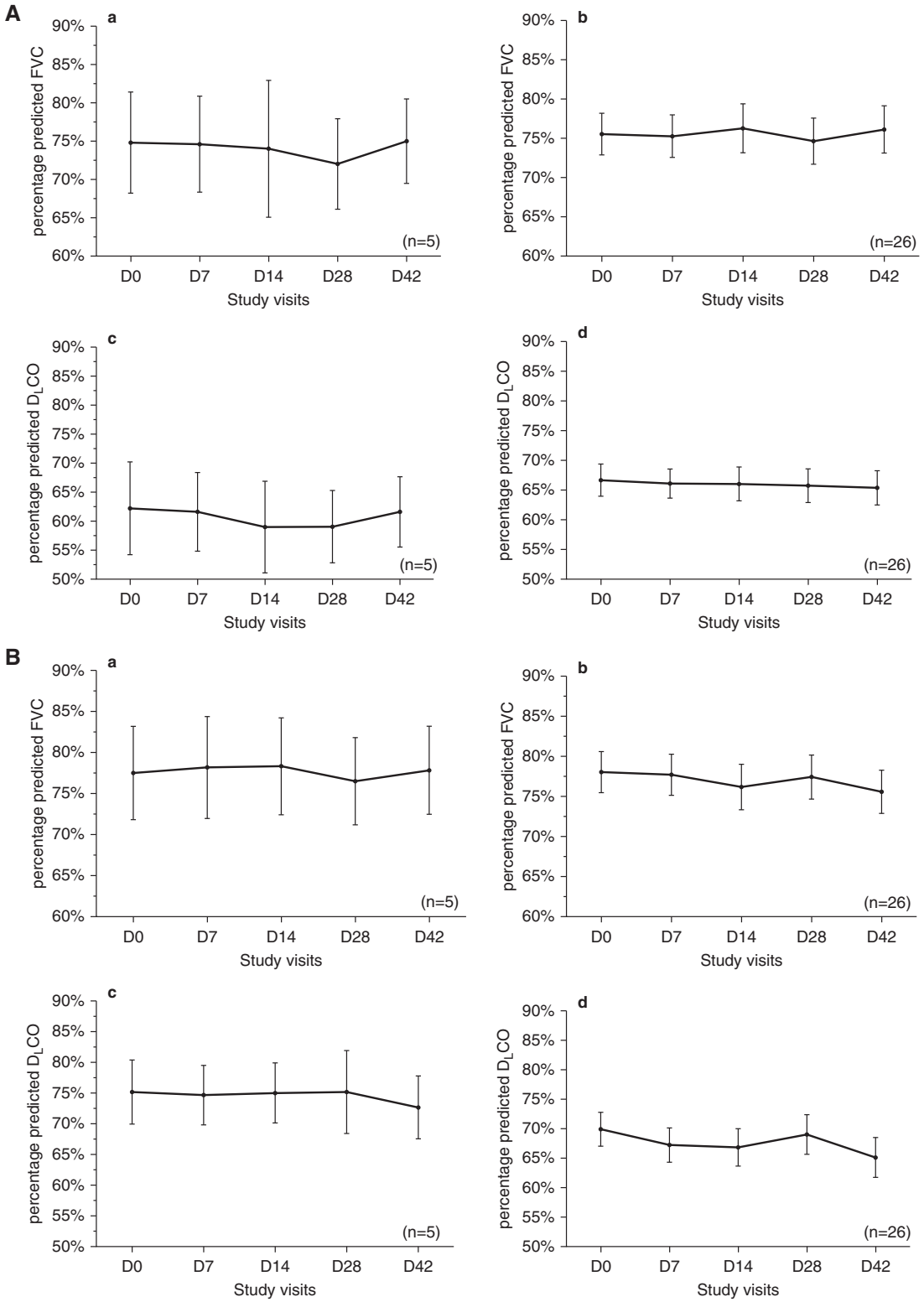

Figure 3. (*A*) Active tuberculosis patients' FVC and D_{LCO} by treatment group over time. Changes in lung function (FVC and D_{LCO}) over the 42-day safety follow-up period are represented as mean and SEM. *a* and *b* depict FVC for the placebo (*a*) and active 3×10^9-vp and 3×10^{10}-vp vaccination (*b*) groups. *c* and *d* depict D_{LCO} for the placebo (*c*) and active 3×10^9-vp and 3×10^{10}-vp vaccination (*d*) groups. (*B*) Posttuberculosis patients' FVC and D_{LCO} by treatment group over time. Changes in lung function (FVC and D_{LCO}) over the 42-day safety period are represented as mean and SEM. *a* and *b* present values for FVC in the placebo (*a*) and active 3×10^9-vp and 3×10^{10}-vp vaccination (*b*) groups. *c* and *d* present values for D_{LCO} in the placebo (*c*) and active 3×10^9-vp and 3×10^{10}-vp vaccination (*d*) groups. D_{LCO} = diffusing capacity of the lung for carbon monoxide; vp = viral particles.

Table 3. Chest Radiographic Changes during the Safety Follow-up Days 7–182

Change in Chest Radiograph	APTB Stratum		PPTB Stratum	
	Vaccinated	Placebo	Vaccinated	Placebo
From Day 7 to Day 14				
Improved	10	1	1	1
Unchanged	19	4	25	5
Worsened	2	0	1	0
Total	31	5	27	6
From Day 14 to Day 42				
Improved	17	3	3	1
Unchanged	7	2	25	5
Worsened	2	0	1	0
Total	26	5	29	6
From Day 42 or 56* to Day 84				
Improved	16	3	2	0
Unchanged	10	1	25	5
Worsened	2	0	1	1
Total	28	4	28	6
From Day 84 to Day 182				
Improved	15	3	3	0
Unchanged	12	2	25	6
Worsened	0	0	0	0
Total	27	5	28	6

Definition of abbreviations: APTB = active pulmonary tuberculosis; PPTB = post-treatment for pulmonary tuberculosis.
*From Day 42 for participants receiving one dose of study vaccine, and from Day 56 for participants receiving two doses of study vaccine.

administered to persons with known pulmonary TB, either actively receiving treatment or after successful treatment. The results confirm the generally favorable safety profile, seen previously in healthy adults and infants (11, 12, 19–21), of the doses tested, which included the highest dose administered on two occasions 6 weeks apart. The special concern, that vaccination might give rise to the Koch phenomenon in participants with heightened immune responsiveness to mycobacterial antigens from current or prior pulmonary TB disease, is not supported.

Although an immune response to the candidate vaccine was demonstrated, greatest in participants receiving the double dose, no acute severe pulmonary AEs were observed at any of the three dose levels used in this study. No clinically significant changes in either lung physiology or radiography were demonstrated, despite evidence of a systemic immunologic response. Only one severe AE (pyrexia) considered vaccine-related was reported, after the first dose at the highest dose level (3×10^{10} vp) in the APTB stratum. However, there was no recurrence after the second dose. All cases of pyrexia resolved spontaneously without sequelae. These findings, along with the absence of any report of clinical symptomatology or measurable pulmonary insufficiency, suggest that administration of up to two doses of 3×10^{10} vp of AERAS-402 to persons receiving treatment for APTB, or previously treated TB, does not raise the specter of precipitating an acute Koch phenomena–like local, pulmonary, or systemic reaction.

Table 4. Comparison of Abnormal Features on Initial (Day 0, Day of Vaccination) and Follow-up (Day 28) Computed Tomography Scan

Overall assessment of change in abnormalities	APTB Stratum		PPTB Stratum	
	Vaccinated	Placebo	Vaccinated	Placebo
Improved	12	2	5	0
Unchanged	14	2	24	6
Worsened	2	1	1	0
Total	28	5	30	6

Definition of abbreviations: APTB = active pulmonary tuberculosis; PPTB = post-treatment for pulmonary tuberculosis.
Consensus report by a pulmonologist and a radiologist.

Although the suggestion of a trend toward worsening in D_{LCO} over 42 days was seen in the APTB stratum receiving the lowest of the three dose levels of AERAS-402, this was unlikely to be vaccine-related given that this was not seen with the two higher dose levels. This likely represents a chance observation. However, in no participants was the deterioration outside the limits of reproducibility for this test over time (≤9%) (18). Overall, the safety results are reassuring, and do not suggest immunologically based deterioration of pulmonary function after vaccination.

There was little difference in the safety endpoints in recipients of AERAS-402 compared with placebo, with the exception of increased reports of hematuria (by dipstick) among vaccine recipients compared with placebo. Reports of hematuria seemed to be dose-related, occurring only at the two highest dose levels of AERAS-402. Hematuria was asymptomatic (no gross hematuria was seen) but formal renal function testing was not performed. In addition, when confounding factors were taken into account (preexisting hematuria, presence of menstruation, an AE reporting period twice as long for the two-dose group, and potential false-positive results with the dipstick test), there was no indication of an excess of hematuria among vaccinated participants compared with placebo. If the hematuria was vaccine related, it is unclear whether it signifies renal pathology, and, if so, what the medium- and long-term consequences of this might be. Focal glomerular damage mediated by immune complex deposition might be considered as the cause for the hematuria, but this remains speculative.

Vaccination with AERAS-402 resulted in the induction of potent and sustained $CD8^+$ and $CD4^+$ T-cell responses to Ag85A and Ag85B. $CD8^+$ responses to the TB10.4 antigen were less pronounced in the PBMC ICS assay. In the whole-blood assay TB10.4-specific responses were of similar magnitude in $CD4^+$ and $CD8^+$ cells. This difference may be accounted for by differences in the duration of antigen stimulation times (6 h vs. 12 h for the PBMC and whole-blood assays, respectively). Our findings are generally

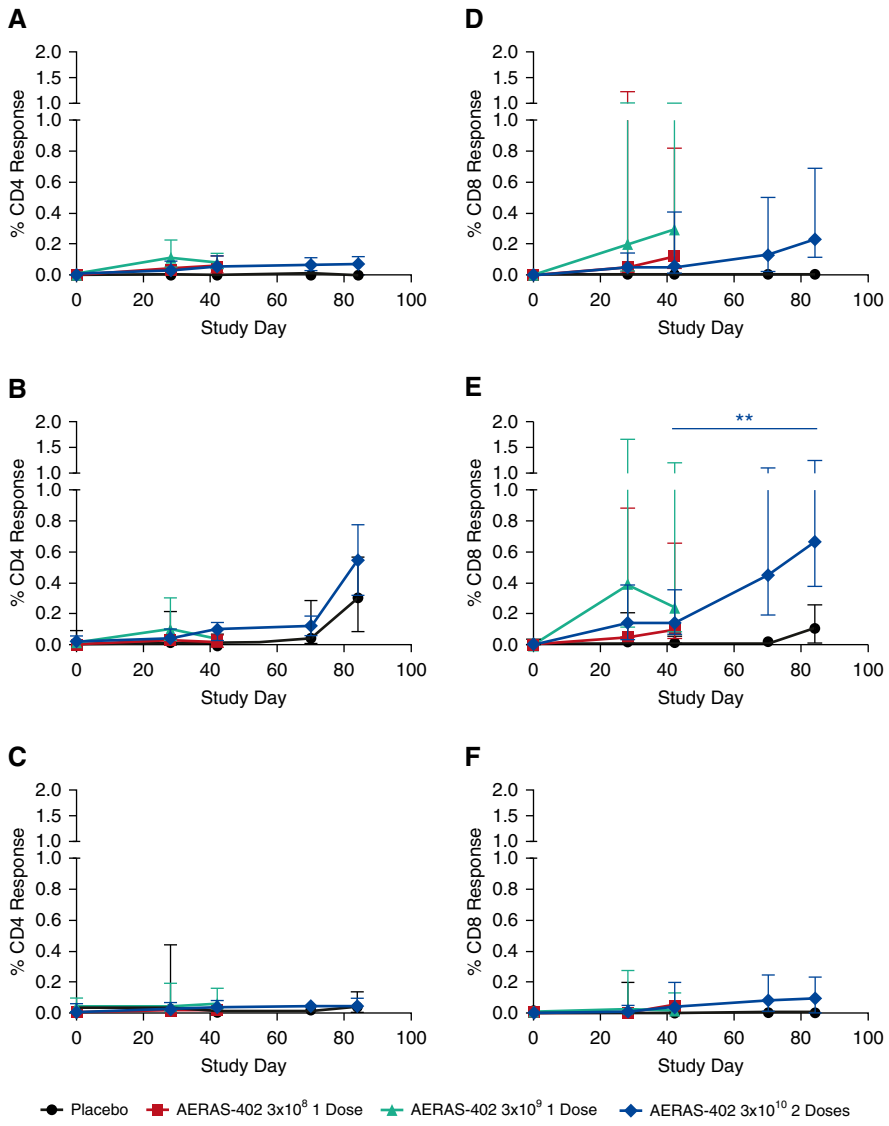

Figure 4. Antigen-specific T-cell responses, expressed as total cytokine-expressing T cells. Peripheral blood mononuclear cells were thawed, rested overnight, and stimulated for 6 hours with peptide pools corresponding to the vaccine antigens Ag85A, Ag85B, and TB10.4. Cells were stained for viability, phenotypic markers, and cytokines (IFN-γ, IL-2, and tumor necrosis factor) and evaluated by flow cytometry. Background-subtracted responses are shown for CD4 (*A–C*) and CD8 (*D–F*) T cells making any combination of cytokines after stimulation with Ag85A (*A* and *D*), Ag85B (*B* and *E*), and TB10.4 (*C* and *F*). *Symbols* and *error bars* represent the median and interquartile range, respectively, for participants receiving one or two doses of placebo (*black circles*), a single dose of AERAS-402 at 3×10^8 vp (*red squares*), 3×10^9 vp (*green triangles*), or two doses of AERAS-402 at 3×10^{10} vp (*blue diamonds*). **Significant increase in response between Days 42 and 84 for the group receiving two doses of AERAS-402 at 3×10^{10} vp ($P = 0.0034$). vp = viral particles.

consistent with prior reports on the use of AERAS-402 in healthy adult and infant populations (11, 19–21). Although the lowest dose induced the lowest frequencies of antigen-specific T cells, the highest dose resulted in a reduction in polyfunctionality of $CD8^+$ T cells (cells producing all three cytokines) compared with the lower doses. The absence of the IL-2–producing $CD8^+$ T cells in the high-dose group suggests that higher doses may not be as effective at inducing long-term $CD8^+$ central memory cells compared with lower doses of AERAS-402. This is consistent with prior research that suggests lower antigen doses may lead to central memory generation, whereas higher doses tend to drive effector/effector memory populations (22, 23).

A second high dose of AERAS-402 seemed to boost the immunologic response, but this was followed by a decrease in responses at later time points, and a switch to a population of $CD8^+$ T cells producing TNF alone (Figure 5B). This is in contrast to a previous study that showed no evidence that two doses of AERAS-402 at 3×10^{10} vp given 56 days apart were more immunogenic than a single dose (8). Although the origins of this new population are unknown, it may be the result of a loss of function of polyfunctional/bifunctional T cells. These data suggest a movement toward less functionality over time and the generation of effector/effector memory T cells. Although the optimal functional cell population for combating TB is not known, effector memory T cells seem to be critical for protection from simian immunodeficiency virus challenge in macaque models, suggesting this population plays an important role in antimicrobial immunity (24). The influence of the presence of Ad35 serum neutralizing activity in 25% of participants at the time of vaccination and subsequent development of activity in 41% of the remainder on these immune responses is unclear.

This study has several limitations. First, this small study cannot ensure the safety of this TB candidate vaccine in a population-wide vaccination program, but as a preliminary evaluation of vaccinating patients with current or recent pulmonary TB, the absence of clinically significant systemic and local detectable adverse reactions is reassuring. Together with data from TB vaccine studies in recipients with latent TB, it paves the way for further larger studies in the general population, without special regard to ensuring that those with prior pulmonary TB exposure are excluded. A further limitation of the study is the relative insensitivity of the methods used to assess events at tissue level in a complex organ like the lungs. Spirometry is predominantly a measure of airway pathology manifesting as airflow limitation. FVC and D_{LCO} were likely to be more useful because they are considered the most sensitive measures of diffuse parenchymal disease of the lung (25–27). However, all are relatively insensitive to regional changes. Thus, large numbers of patients would be required to detect small changes in lung function. However, no trends were

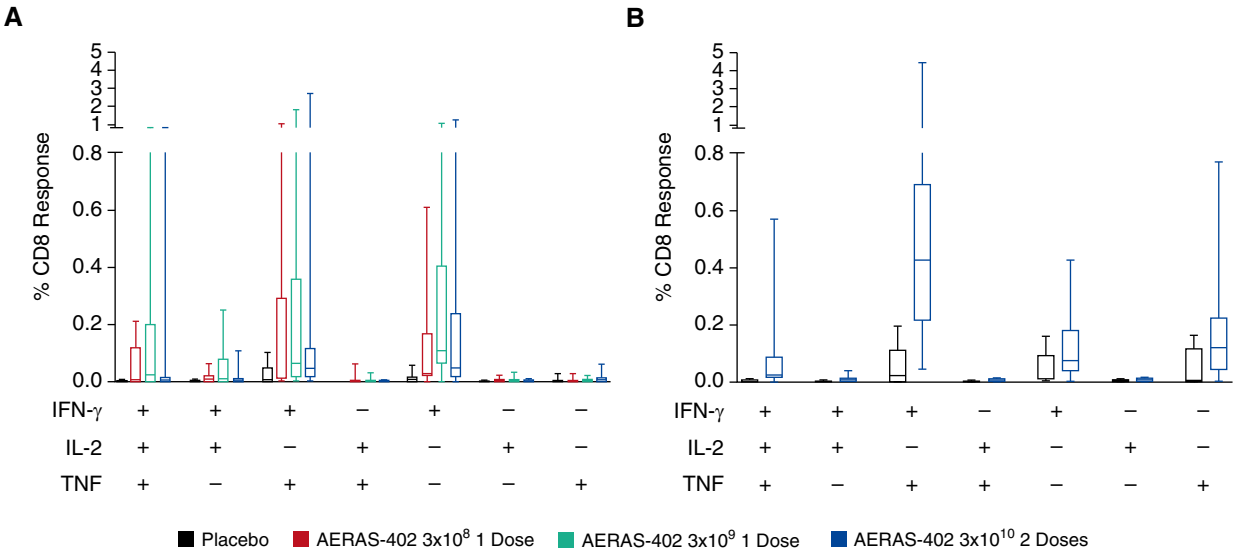

Figure 5. Cytokine coexpression patterns by Ag85B-specific CD8 T cells on Days 42 and 84. Peripheral blood mononuclear cells were thawed, rested overnight, and stimulated for 6 hours with peptide pools corresponding to the vaccine antigens Ag85A, Ag85B, and TB10.4. Cells were stained for viability, phenotypic markers, and cytokines (IFN-γ, IL-2, and tumor necrosis factor) and evaluated by flow cytometry. Background-subtracted responses are plotted for $CD8^+$ T cells after stimulation with Ag85B for Days 42 (single dose, *A*) and 84 (two doses, *B*). Only the two-dose placebo and high-dose groups (3×10^{10} vp) were followed out to Day 84. *Box-and-whisker plots* represent group interquartile ranges and minimum/maximum for participants receiving one or two doses of placebo (*black*), a single dose of AERAS-402 at 3×10^8 vp (*red*) or 3×10^9 vp (*green*), or two doses of AERAS-402 at 3×10^{10} vp (*blue*). *Horizontal lines* represent group median responses. TNF = tumor necrosis factor; vp = viral particles.

evident in this study, which was of adequate duration to examine short- and longer-term adverse outcomes in the lung.

High-resolution CT scans are considered the most sensitive method for detecting local or segmental changes in diseased lungs, and are increasingly being used to monitor changes in other lung diseases, particularly in patients with diffuse parenchymal diseases. Their use is limited by concerns about radiation, and it is difficult to justify more than three scans per patient when performed for research purposes alone. The limited low-dose scanning protocol used in our study, however, was considered safe. For future studies, new ultra-low-dose chest CT technology, supplemented with automated registration to compare corresponding images in different scans, will be a major advance and will make this method more acceptable, providing better detection of even small changes in areas of tuberculous disease in the lung.

Conclusions

This is the one of the first studies to formally investigate the effects a candidate vaccine against *M.tb* in participants with known active or previous pulmonary TB. Our findings demonstrate that the AERAS-402 candidate vaccine can be safely administered to individuals on treatment, or who have recently completed treatment, for APTB. No paradoxical reactions, specifically acute, Koch phenomenon–like reactions, were seen. AERAS-402 was immunogenic and the highest magnitude of response was noted after a booster administered 42 days after the priming dose. Because the numbers enrolled in this study were small, future studies of highly immunogenic TB vaccine candidates need to include careful assessments of pulmonary or systemic AEs if administered to individuals with APTB or PPTB. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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